

mRNA -54 ACTTTGAAGTCTCTTAATTTGTTCCCTCGTAGAAAGAACGCATAGATAATT -5
|||||
gDNA ACTTTGAAGTCTCTTAATTTGTTCCCTCGTAGAAAGAACGCATAGATAATT
-4 CAAAATGG 4
|||||
CAAAATGGTATGTGTTTATAGTTCATGTGCCGAACAACACCGTT
5' ss
5 TTAACCTCACTGTCG 19
|||||
TCAAAGATGGGAGCCAGCCACTAAACATCTCCTCTAGTTAACTTCACTGTCG
3' ss
20 ATCAGATGCGATCCCTTATGGACAAGGTGTCCAAACGTCCGTAACATGTCG 69
|||||
ATCAGATGCGATCCCTTATGGACAAGGTGACCAACGTCCGTAACATGTCG
70 GTTATTGCCCCACGTTGATCAGGGTAAGTCCACTTTAACTGACTCCCCTGGT 119
|||||
GTTATTGCCCCACGTTGATCAGGGTAAGTCCACTTTAACTGACTCCCCTGGT

FIG. 2

-51 -41 -31 -21 -11 -1 GATACCTT TGAAGTCTCT AATTTTGTTCT CTCGTAGAAA GAACGCATAG ATAATTCAAA																	
9			18			27			36			45			54		
ATG	GTT	AAC	TTC	ACT	GTC	GAT	CAG	ATG	CGA	TCC	CTT	ATG	GAC	AAG	GTG	ACC	AAC
M	V	N	F	T	V	D	Q	M	R	S	L	M	D	K	V	T	N
63			72			81			90			99			108		
GTC	CGT	AAC	ATG	TCG	GTT	ATT	GCC	CAC	GTT	GAT	CAC	GGT	AAG	TCC	ACT	TTA	ACT
V	R	N	M	S	V	I	A	H	V	D	H	G	K	S	T	L	T
117			126			135			144			153			162		
GAC	TCC	CTG	GTG	CAA	CGT	GCC	GGT	ATT	ATT	TCT	GCT	GCC	AAG	GCT	GGT	GAG	GCC
D	S	L	V	Q	R	A	G	I	I	S	A	A	K	A	G	E	A
171			180			189			198			207			216		
CGT	TTC	ACT	GAT	ACT	AGA	AAG	GAC	GAG	CAA	GAG	AGA	GGT	ATC	ACC	ATC	AAG	TCT
R	F	<u>T</u>	D	T	R	K	D	E	Q	E	R	G	I	T	I	K	S
225			234			243			252			261			270		
ACC	GCC	ATT	TCT	TTG	TAC	TCT	GAG	ATG	GGT	GAC	GAC	GAT	GTC	AAG	GAG	ATC	AAG
<u>T</u>	<u>A</u>	I	S	L	Y	S	E	M	G	D	D	D	V	K	E	I	K
279			288			297			306			315			324		
CAG	AAG	ACT	GAA	GGT	AAC	AGT	TTC	CTT	ATC	AAC	TTA	ATT	GAC	TCC	CCA	GGT	CAC
Q	K	T	E	G	N	S	F	L	I	N	L	I	D	S	P	G	H
333			342			351			360			369			378		
GTT	GAC	TTC	TCT	TCT	GAG	GTC	ACT	GCT	GCT	CTG	CGT	GTT	ACT	GAC	GGT	GCT	TTG
V	D	F	S	S	E	V	T	A	A	L	R	V	T	D	G	A	L
387			396			405			414			423			432		
GTC	GTC	GTT	GAC	TGT	GTT	GAA	GGT	GTC	TGT	GTT	CAA	ACT	GAG	ACC	GTT	TTG	CGT
V	V	V	D	C	V	E	G	V	C	V	Q	T	E	T	V	L	R
441			450			459			468			477			486		
CAA	GCT	TTG	GGT	GAA	AGA	ATC	AAG	CCA	GTT	GTT	GTC	ATT	AAC	AAG	GTC	GAC	CGT
Q	A	L	G	E	R	I	K	P	V	V	V	I	N	K	V	D	R
495			504			513			522			531			540		
GCT	CTT	TTG	GAG	TTG	CAA	GTT	ACC	AAG	GAG	GAC	CTG	TAC	CAG	TCT	TTC	GCT	AGA
A	L	L	E	L	Q	V	T	K	E	D	L	Y	Q	S	F	A	R
549			558			567			576			585			594		
ACC	GTC	GAG	TCC	GTA	AAC	GTC	GTT	ATC	GCT	ACT	TAC	ACT	GAC	AAG	ACC	ATT	GGT
T	V	E	S	V	N	V	V	I	A	T	Y	T	D	K	T	I	G
603			612			621			630			639			648		
GAC	AAC	CAA	GTC	TAC	CCA	GAA	CAG	GGT	ACC	GTC	GCT	TTC	GGT	TCA	GGT	CTG	CAC
D	N	Q	V	Y	P	E	Q	G	T	V	A	F	G	S	G	L	H
657			666			675			684			693			702		
GGA	TGG	GCT	TTC	ACC	GTT	AGA	CAG	TTC	GCC	ACT	AGA	TAC	TCC	AAG	AAG	TTC	GGT
G	W	A	F	T	V	R	Q	F	A	T	R	Y	S	K	K	F	G

FIG. 3A

GTT	GAC	AGA	ATC	AAG	ATG	ATG	GAG	CGT	CTG	TGG	GGA	GAC	TCT	TAC	TTC	AAC	CCA
V	D	R	I	K	M	M	E	R	L	W	G	D	S	Y	F	N	P
AAG	ACC	AAG	AAA	TGG	ACC	AAC	AAG	GAC	AAG	GAC	GCC	GCT	GGA	AAG	CCT	TTG	GAG
K	T	K	K	W	T	N	K	D	K	D	A	A	G	K	P	L	E
CGT	GCC	TTC	AAC	ATG	TTC	GTT	TTG	GAC	CCT	ATC	TTC	CGT	CTG	TTT	GCT	GCC	ATC
R	A	F	N	M	F	V	L	D	P	I	F	R	L	F	A	A	I
ATG	AAC	TTC	AAG	AAG	GAT	GAA	ATT	CCA	GTT	CTG	TTG	GAG	AAA	TTG	GAG	ATC	AAC
M	N	F	K	K	D	E	I	P	V	L	L	E	K	L	E	I	N
CTG	AAG	CGT	GAG	GAG	AAG	GAG	TTG	GAG	GGT	AAG	GCT	CTT	TTG	AAG	GTT	GTC	ATG
L	K	R	E	E	K	E	L	E	G	K	A	L	L	K	V	V	M
AGA	AAG	TTC	TTG	CCA	GCT	GCC	GAC	GCT	TTG	TTG	GAG	ATG	ATT	GTT	CTT	CAC	CTG
R	K	F	L	P	A	A	D	A	L	L	E	M	I	V	L	H	L
CCA	TCT	CCA	GTC	ACC	GCT	CAA	GCT	TAC	AGA	GCC	GAG	ACT	TTG	TAC	GAA	GGT	CCA
P	S	P	V	T	A	Q	A	Y	R	A	E	T	L	Y	E	G	P
TCT	GAT	GAC	CAA	TTC	TGC	ATT	GGT	ATC	AGA	GAG	TGT	GAC	CCT	AAG	GCT	GAG	CTG
S	D	D	Q	F	C	I	G	I	R	E	C	D	P	K	A	E	L
ATG	GTT	TAC	ATT	TCC	AAG	ATG	GTG	CCA	ACC	TCC	GAC	AAA	GGT	AGA	TTC	TAC	GCC
M	V	Y	I	S	K	M	V	P	T	S	D	K	G	R	F	Y	A
TTC	GGT	CGT	GTT	TTC	TCC	GGT	ACT	GTT	AAG	TCC	GGT	CAA	AAG	GTC	AGA	ATC	CAA
F	G	R	V	F	S	G	T	V	K	S	G	Q	K	V	R	I	Q
GGT	CCT	AAC	TAC	GTT	CCA	GGT	AAG	AAG	GAG	GAC	TTG	TTC	ATC	AAG	GCT	GTT	CAA
G	P	N	Y	V	P	G	K	K	E	D	L	F	I	K	A	V	Q
AGA	ACT	GTT	TTG	ATG	ATG	GGA	AGA	ACC	GTC	GAG	CCT	ATT	GAC	GAT	GTC	CCA	GCT
R	T	V	L	M	M	G	R	T	V	E	P	I	D	D	V	P	A
GGT	AAC	ATT	CTG	GGT	ATT	GTG	GGT	ATC	GAC	CAG	TTC	TTG	CTG	AAG	TCT	GGT	ACT
G	N	I	L	G	I	V	G	I	D	Q	F	L	L	K	S	G	T
CTT	ACT	ACC	AAC	GAA	GCC	GCT	CAC	AAC	ATG	AAG	GTG	ATG	AAA	TTC	TCT	GTC	TCT
L	T	T	N	E	A	A	H	N	M	K	V	M	K	F	S	V	S

$\Delta 5'EF-2$
 1413 1422 1431 1440 1449 1458

FIG. 3B

1467	1476	1485	1494	1503	1512
CCA GTT GTG CAA GTT GCC GTT GAG GTC AAG AAC GCT AAT GAT CTG CCC AAG TTG					
P V V Q V A V E V K N A N D L P K L					
1521	1530	1539	1548	1557	1566
GTT GAG GGT CTG AAG CGT TTG TCC AAG TCT GAC CCA TGT GTT TTA ACC TAC ATC					
V E G L K R L S K S D P C V L T Y I					
1575	1584	1593	1602	1611	1620
TCC GAG TCT GGT GAG CAC ATT GTT GCT GGT ACT GGT GAG CTG CAC TTG GAA ATC					
S E S G E H I V A G T G E L H L E I					
1629	1638	1647	1656	1665	1674
TGT TTG CAA GAT CTG CAA GAC GAC CAC GCT GGT GTC CCT CTG AAG ATT TCT CCT					
C L Q D L Q D D H A G V P L K I S P					
1683	1692	1701	1710	1719	1728
CCA GTT GTT ACC TAC CGT GAG ACT GTC ACT AAC GAA TCT TCC ATG ACT GCC CTG					
P V V T Y R E T V T N E S S M T A L					
1737	1746	1755	1764	1773	1782
TCC AAG TCT CAG AAC AAG CAT AAC AGA ATT TAC CTG AAG GCT CAA CCA ATT GAC					
S K S Q N K H N R I Y L K A Q P I D					
1791	1800	1809	1818	1827	1836
GAG GAA TTG TCT TTG GCT ATC GAA GAA GGT AAG GTT CAC CCA AGA GAC GAC TTT					
E E L S L A I E E G K V H P R D D F					
1845	1854	1863	1872	1881	1890
AAA GCC AGA GCC AGA ATC ATG GCT GAT GAA TAC GGT TGG GAC GTC ACT GAT GCC					
K A R A R I M A D E Y G W D V T D A					
1899	1908	1917	1926	1935	1944
AGA AAG ATC TGG TGT TTC GGT CCA GAC GGT ACT GGT GCC AAC TTA GTT GTT GAC					
R K I W C F G P D G T G A N L V V D					
1953	1962	1971	1980	1989	1998
CAG TCT AAG GCT GTC CAA TAC TTG CAC GAG ATC AAG GAC TCT GTT GTT GCC GGT					
Q S K A V Q Y L H E I K D S V V A G					
2007	2016	2025	2034	2043	2052
TTC CAA TTG GCT ACC AAG GAA GGT CCA ATT TTG GGA GAA AAC ATG AGA TCC GTC					
F Q L A T K E G P I L G E N M R S V					
2061	2070	2079	2088	2097	2106
AGA GTC AAC ATC TTG GAT GTT ACC CTG CAC GCC GAT GCT ATC CAC AGA GGT GGA					
R V N I L D V T L H A D A I H R G G					
2115	2124	2133	2142	2151	2160
GGA CAA GTC ATT CCA ACC ATG AAG AGA GTT ACC TAC GCC GCC TTC CTG TTG GCT					
G Q V I P T M K R V T Y A A F L L A					
2169	2178	2187	2196	2205	2214
GAG CCA GCT ATC CAG GAG CCT ATC TTC TTG GTG GAG ATC CAA TGT CCA GAG AAT					
E P A I Q E F I F L V E I Q C P E N					

FIG. 3C

2223	2232	2241	2250	2259	2268
GCC ATT GGT GGT ATC TAC TCT GTT TTG AAC AAG AAG AGA GGT CAA GTT ATC TCT					
A I G G I Y S V L N K K R G Q V I S					
2277	2286	2295	2304	2313	2322
GAG GAA CAA AGA CCA GGT ACC CCA TTG TTC ACT GTC AAA GGT TAC TTG CCA GTT					
E E Q R P G T P L F T V K A Y L P V					
2331	2340	2349	2358	2367	2376
AAC GAG TCA TTC GGT TTC ACC GGT GAA CTG AGA CAA GCT ACC GCT GGT CAA GCT					
N E S F G F T G E L R Q A T A G Q A					
2385	2394	2403	2412	2421	2430
TTC CCA CAG ATG GTG TTC GAC CAC TGG GCC AAC ATG AAT GGT AAC CCA TTG GAC					
F P Q M V F D H W A N M N G N P L D					
2439	2448	2457	2466	2475	2484
CCA GCC TCC AAG GTC GGT GAG ATT GTT CTT GCT GCC AGA AAG AGA CAG GGT ATG					
P A S K V G E I V L A A R K R Q G M					
2493	2502	2511	2520	2529	2538
AAG GAG AAC GTT CCT GGT TAT GAA GAG TAC TAC GAC AAG TTG TAA GCT TAA TGT					
K E N V P G Y E E Y Y D K L					
2547	2556	2565	2574	2583	2592
TTC ATT AAC TTA TTT GTG TCG TTC GTA TGT CTA TTT ACG TAC TTA ATT CAG TGT					
AS'EF-24					

2601
ATT GTT GTT 3'

FIG. 3D

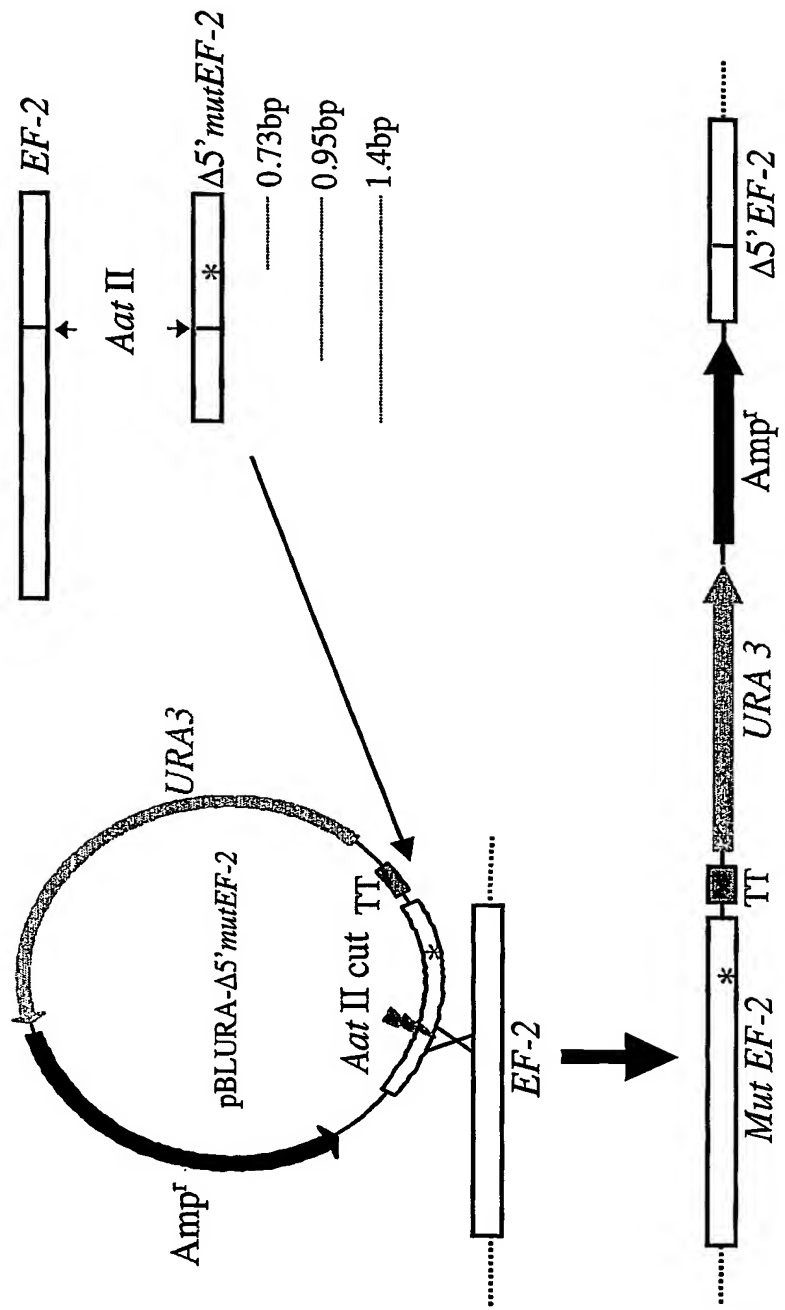


FIG. 4

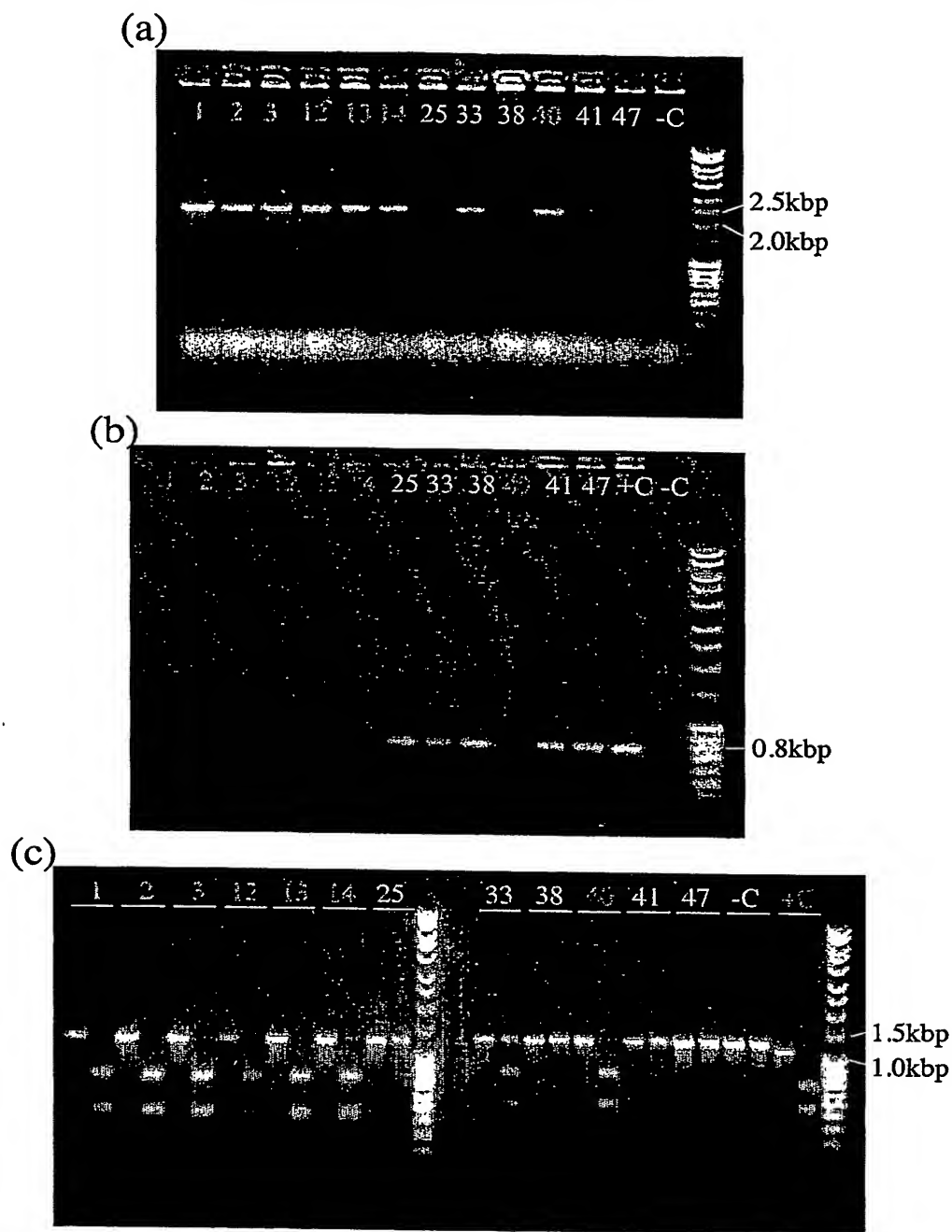


FIG. 5

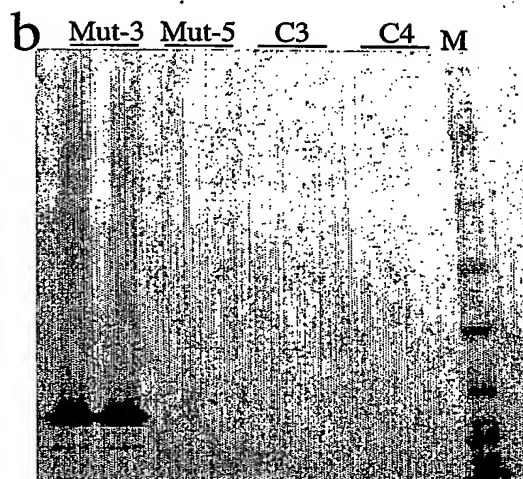
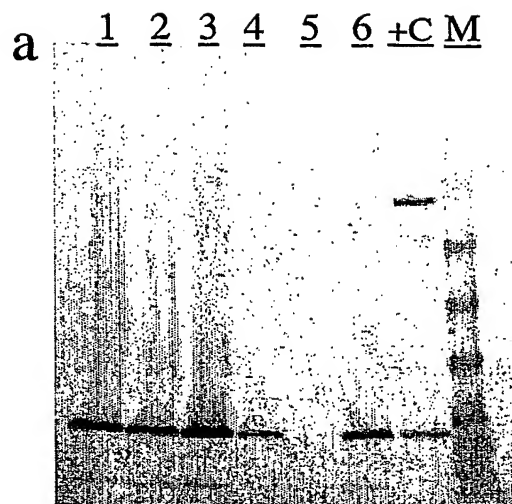


FIG. 6

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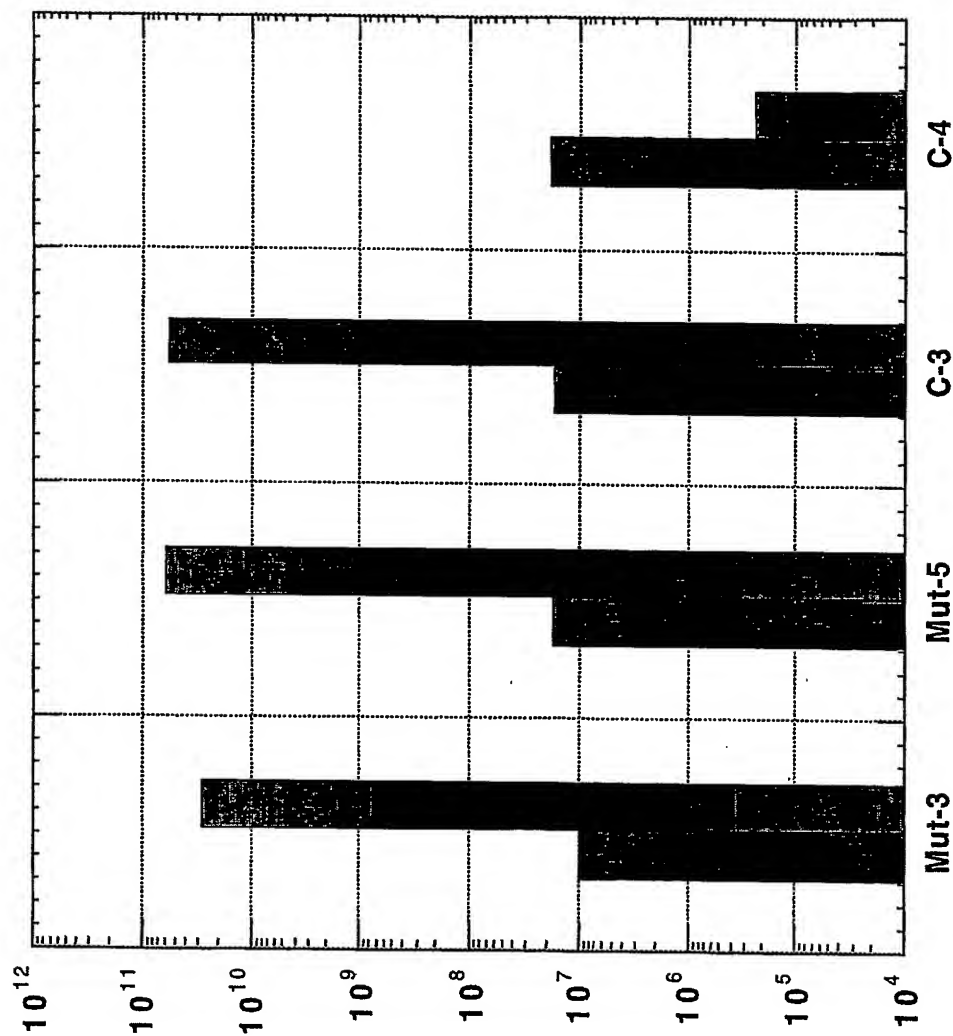


FIG. 7

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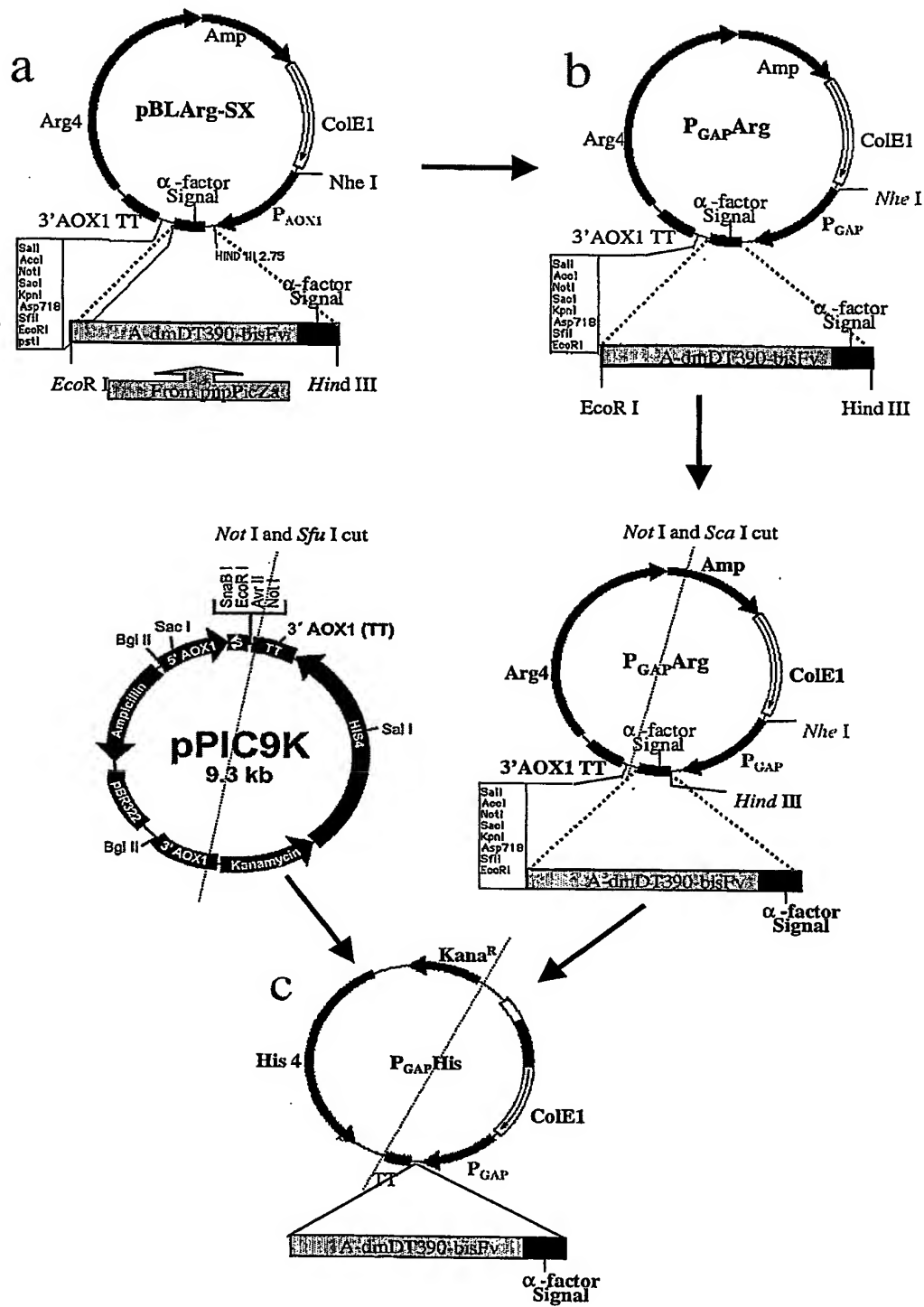


FIG. 8

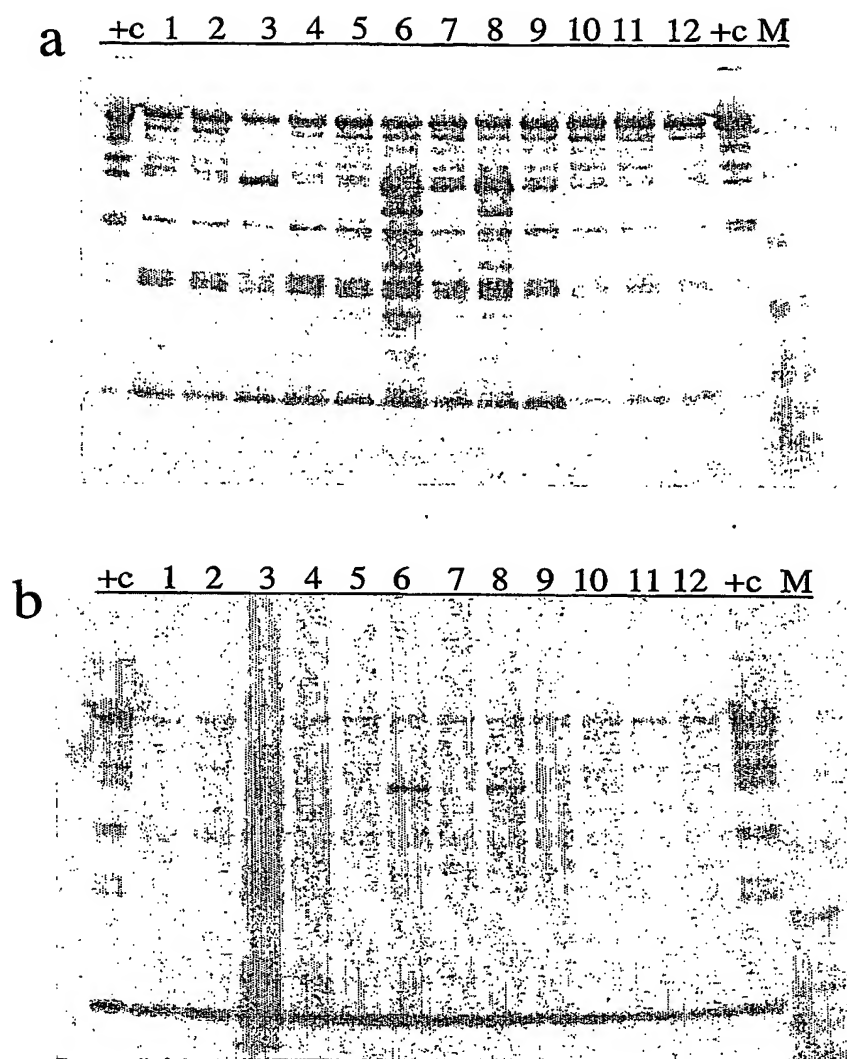


FIG. 9

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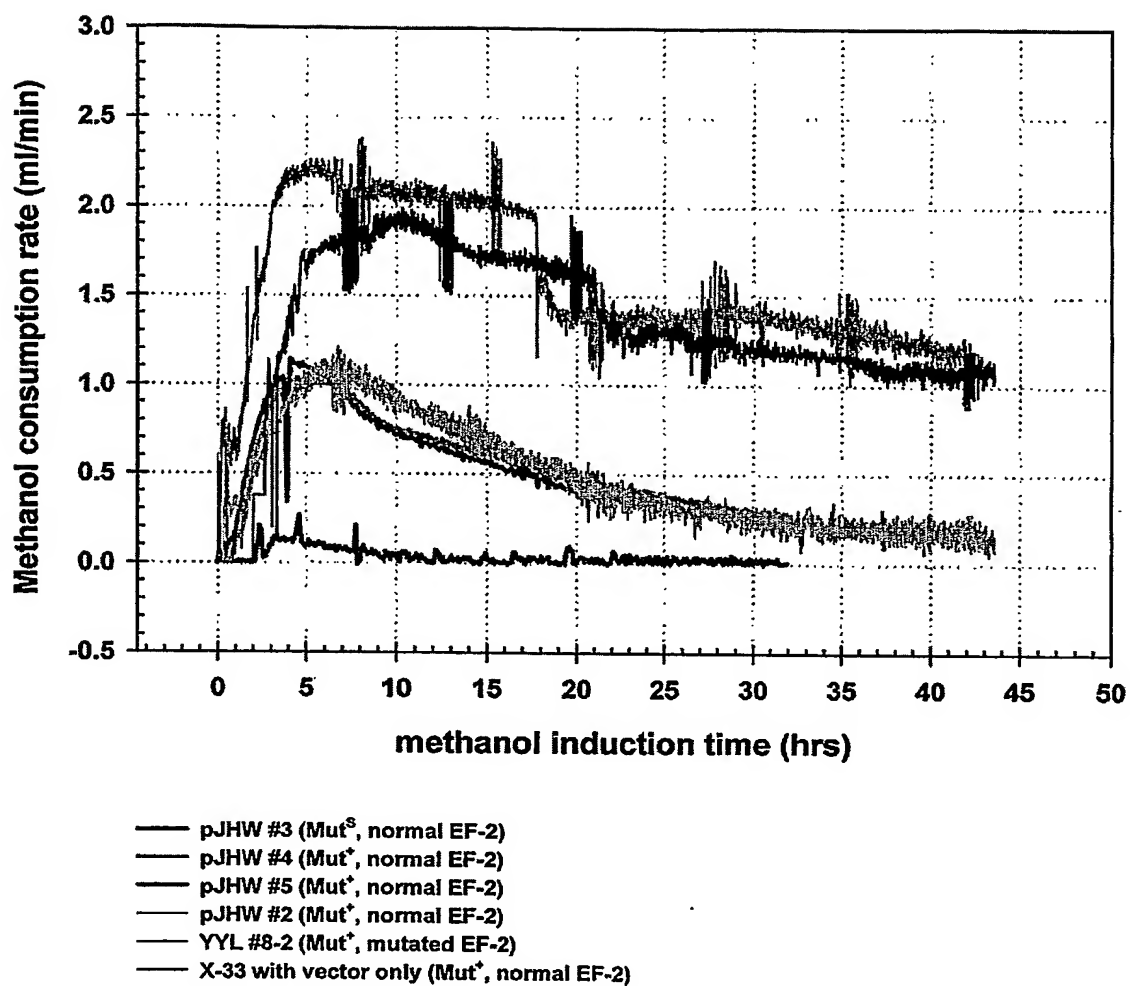


FIG. 10

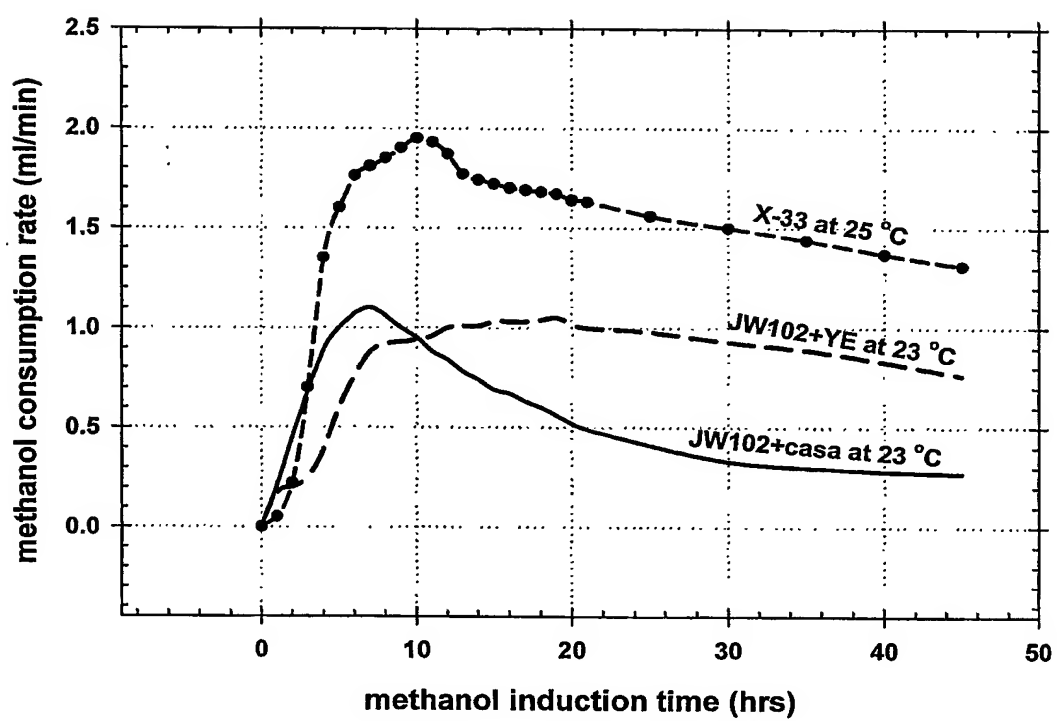


FIG. 11

**Lowering agitation speed in fermentation
reduces immunotoxin aggregates**

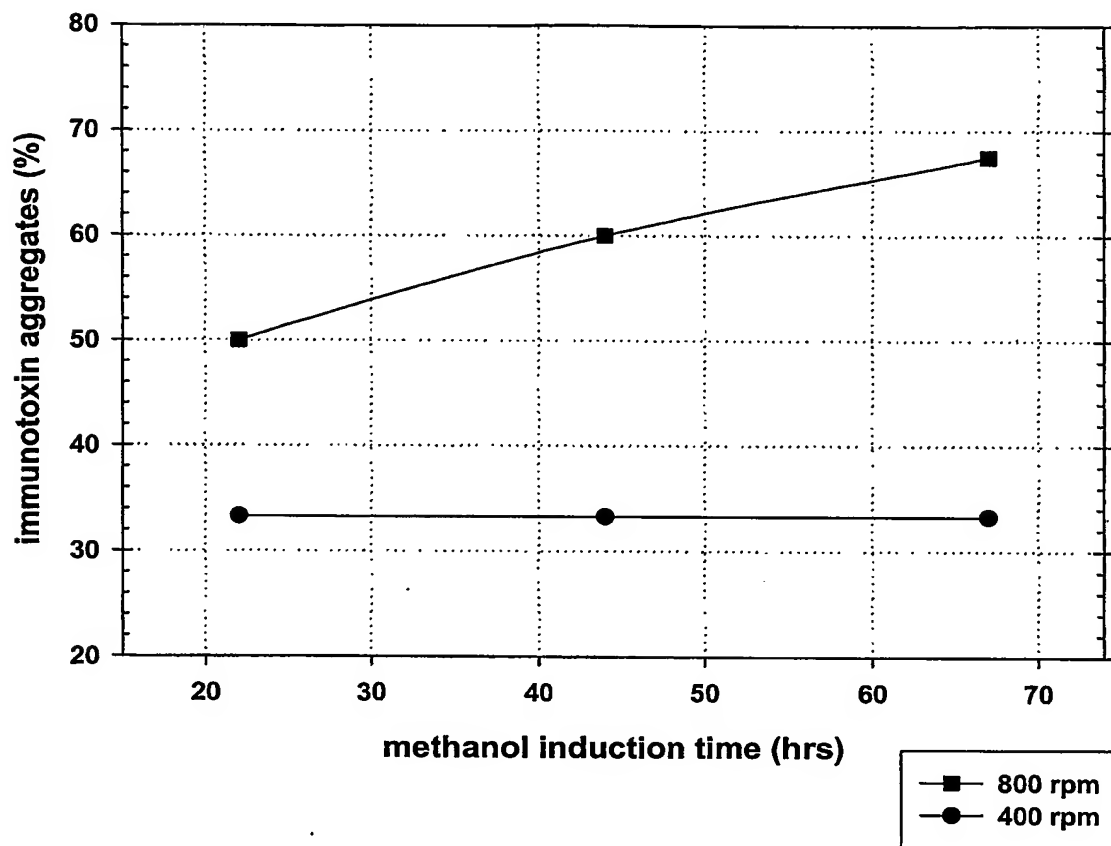


Fig. 12

**Effect of Tween 20 on aggregation of purified immunotoxin
after 20 hrs incubation at 30 C at 250 rpm**

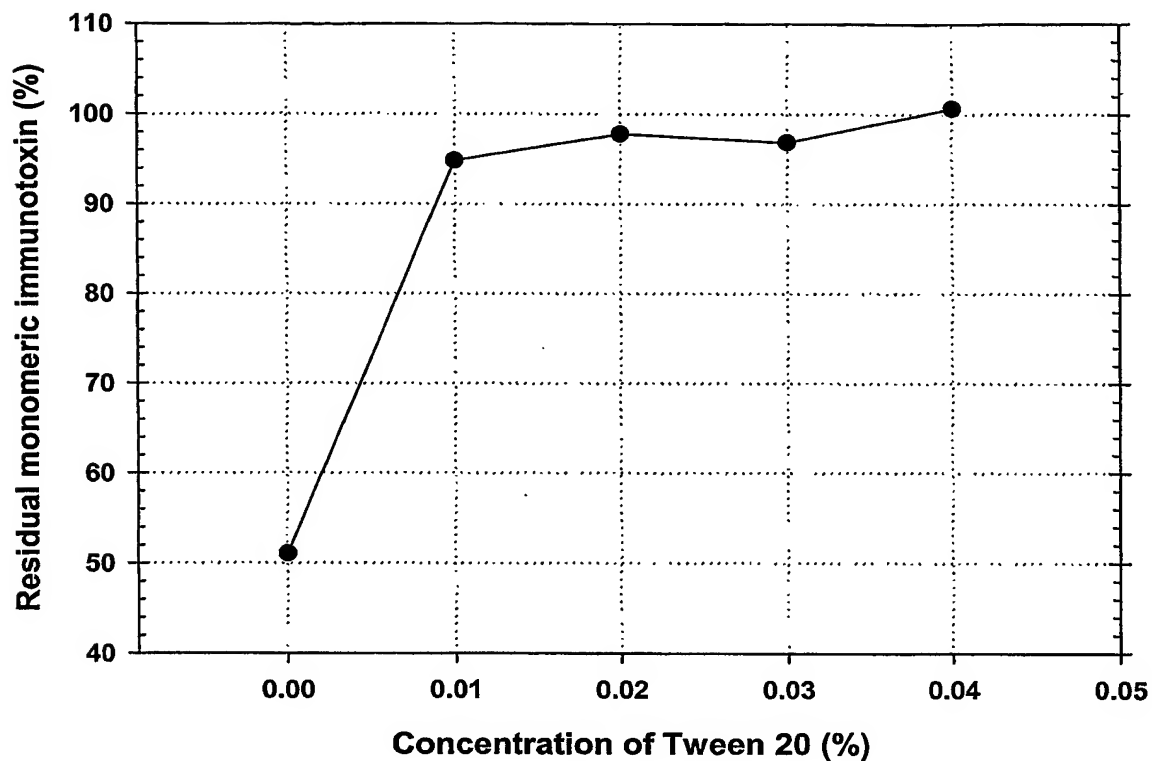


Fig. 13

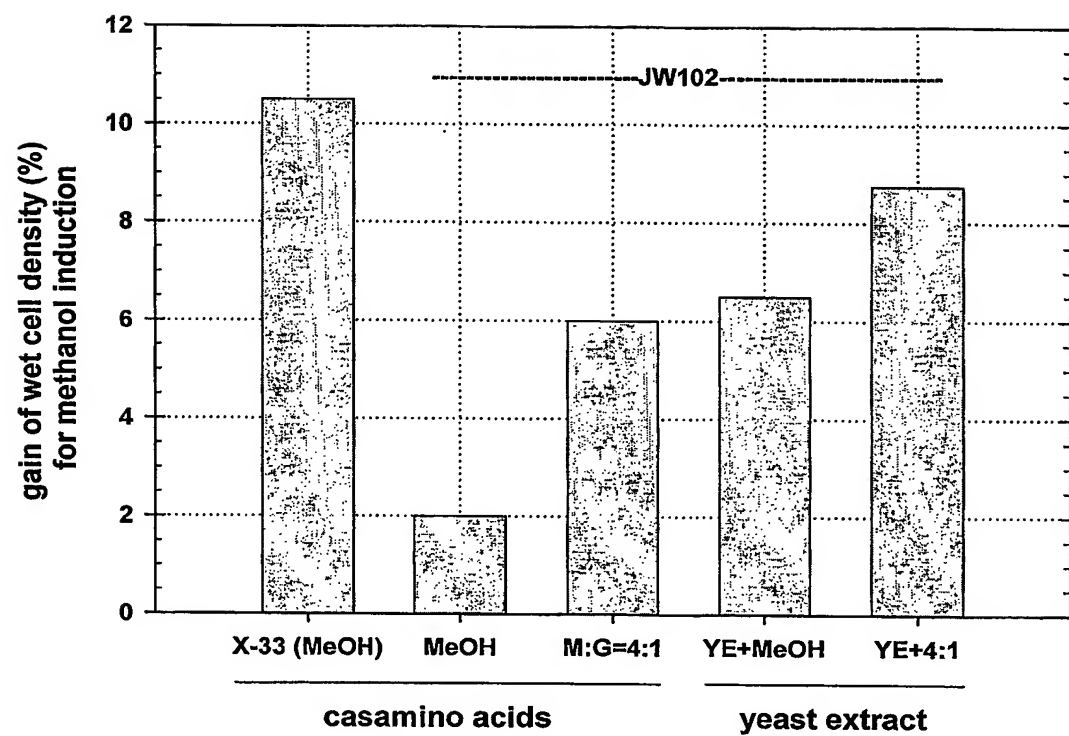


FIG. 14

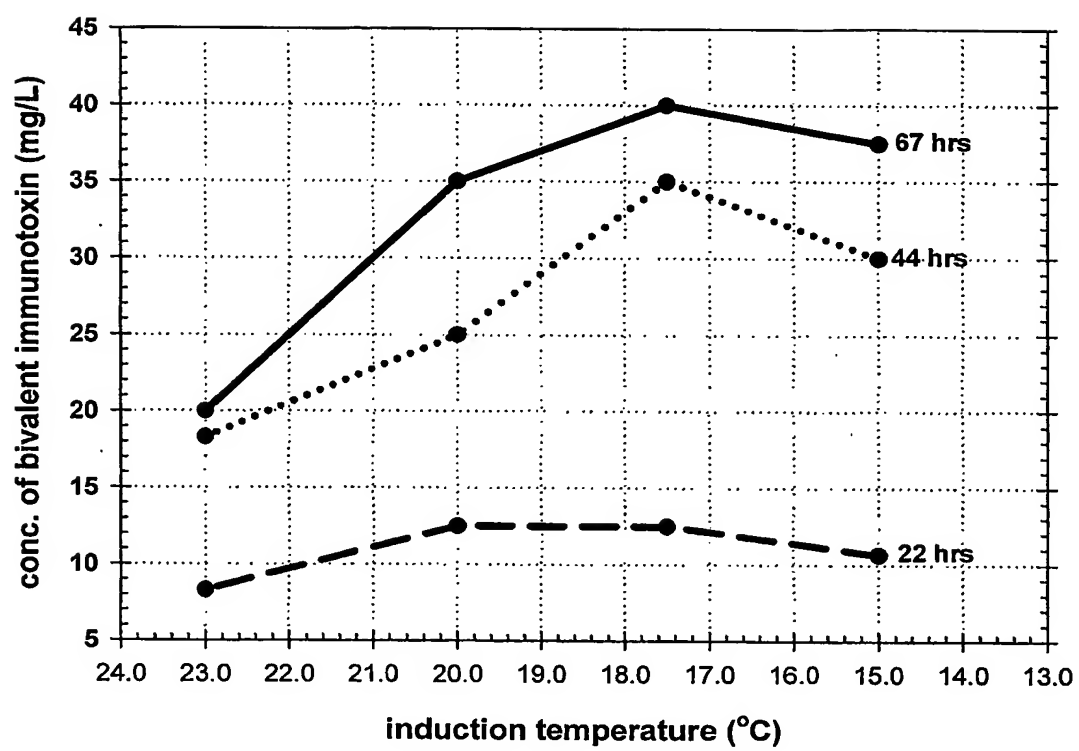


FIG. 15A

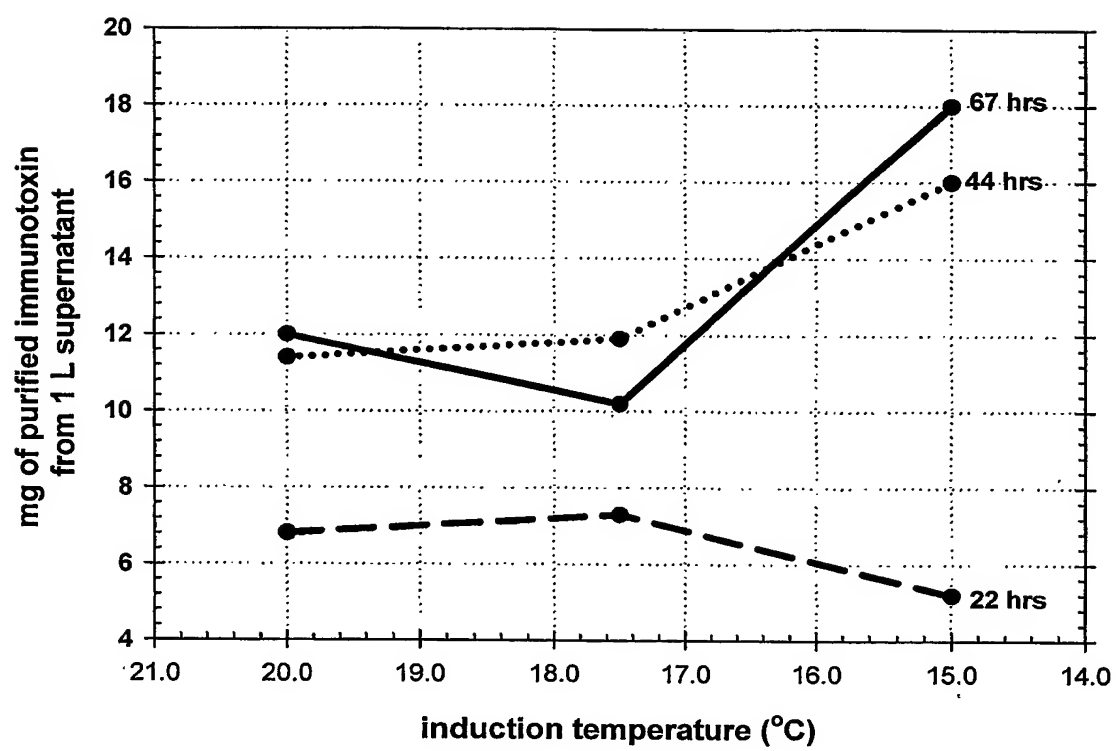


FIG. 15B

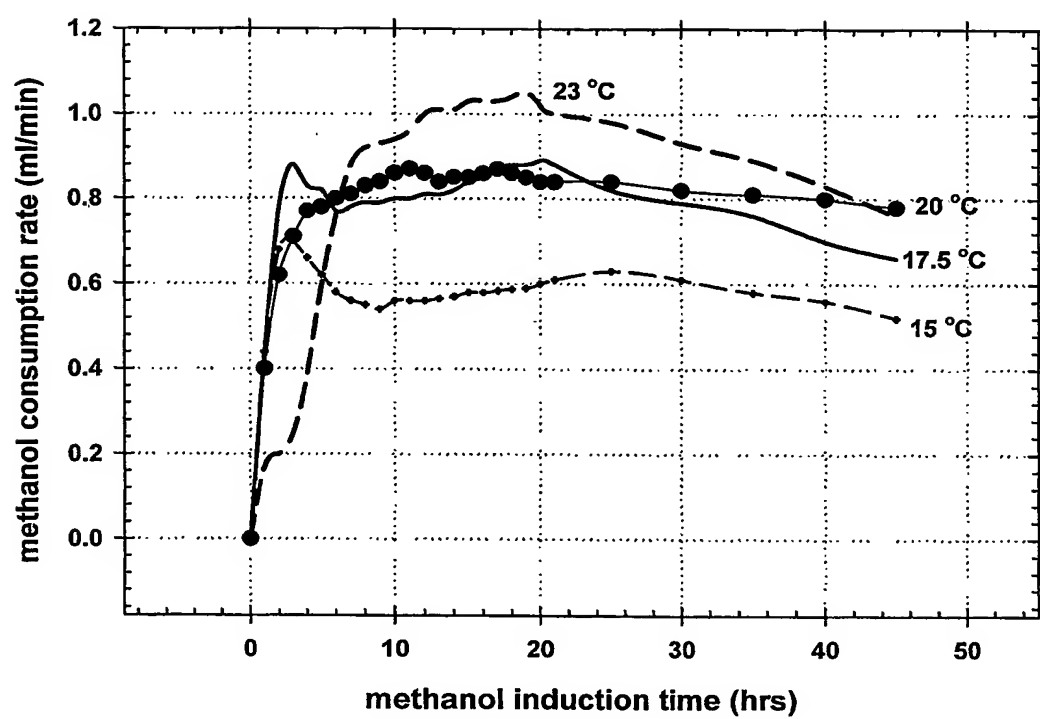


FIG. 15C

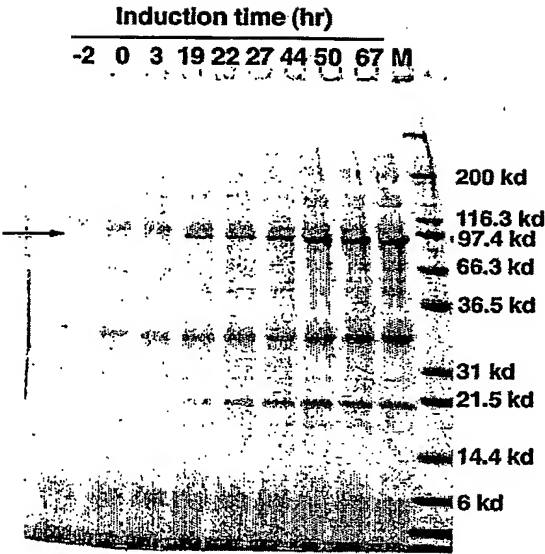


FIG. 16

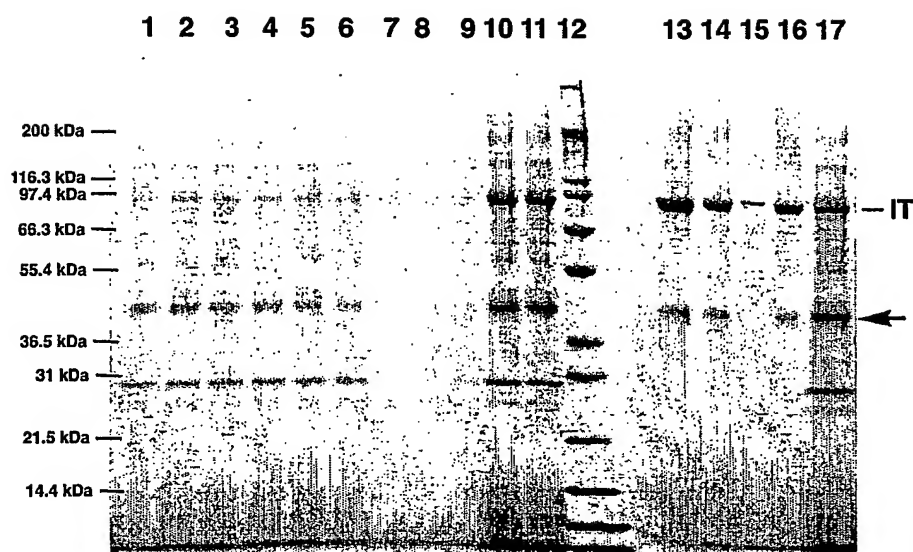


FIG. 17

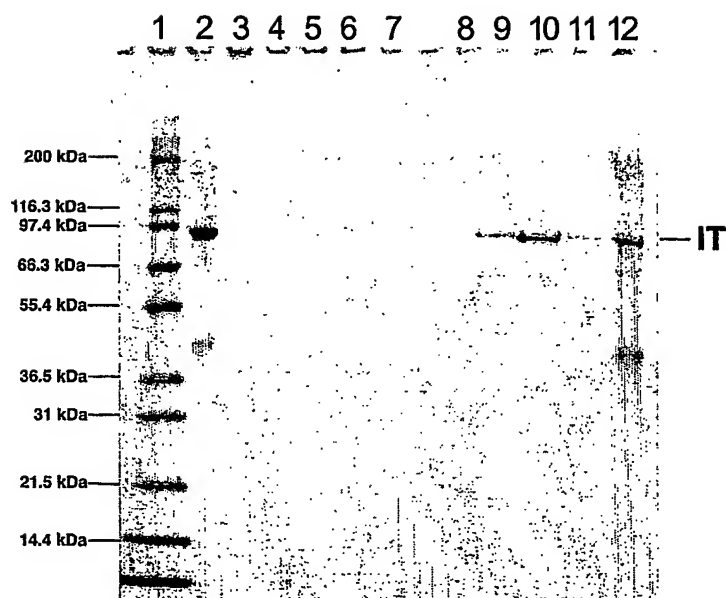
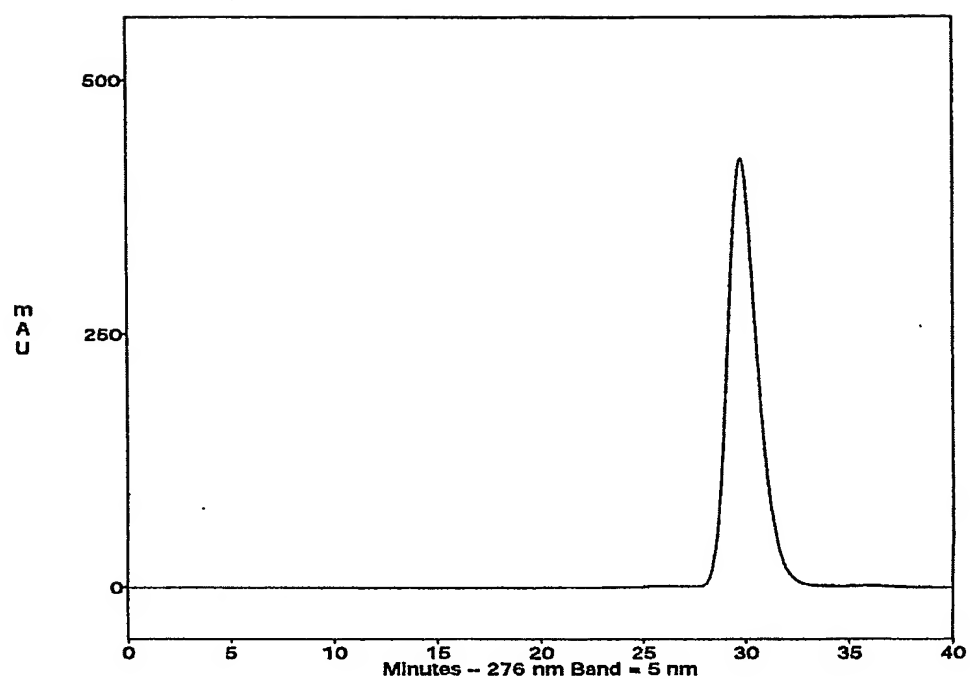


FIG. 18

A



B



FIG. 19

Ala Gly Ala Asp Asp Val Val Asp Ser Ser Lys Ser Phe Val Met Glu
 1 5 10 15
 Asn Phe Ala Ser Tyr His Gly Thr Lys Pro Gly Tyr Val Asp Ser Ile
 20 25 30
 Gln Lys Gly Ile Gln Lys Pro Lys Ser Gly Thr Gln Gly Asn Tyr Asp
 35 40 45
 Asp Asp Trp Lys Gly Phe Tyr Ser Thr Asp Asn Lys Tyr Asp Ala Ala
 50 55 60
 Gly Tyr Ser Val Asp Asn Glu Asn Pro Leu Ser Gly Lys Ala Gly Gly
 65 70 75 80
 Val Val Lys Val Thr Tyr Pro Gly Leu Thr Lys Val Leu Ala Leu Lys
 85 90 95
 Val Asp Asn Ala Glu Thr Ile Lys Lys Glu Leu Gly Leu Ser Leu Thr
 100 105 110
 Glu Pro Leu Met Glu Gln Val Gly Thr Glu Glu Phe Ile Lys Arg Phe
 115 120 125
 Gly Asp Gly Ala Ser Arg Val Val Leu Ser Leu Pro Phe Ala Glu Gly
 130 135 140
 Ser Ser Ser Val Glu Tyr Ile Asn Asn Trp Glu Gln Ala Lys Ala Leu
 145 150 155 160
 Ser Val Glu Leu Glu Ile Asn Phe Glu Thr Arg Gly Lys Arg Gly Gln
 165 170 175
 Asp Ala Met Tyr Glu Tyr Met Ala Gln Ala Cys Ala Gly Asn Arg Val
 180 185 190
 Arg Arg Ser Val Gly Ser Ser Leu Ser Cys Ile Asn Leu Asp Trp Asp
 195 200 205
 Val Ile Arg Asp Lys Thr Lys Thr Lys Ile Glu Ser Leu Lys Glu His
 210 215 220
 Gly Pro Ile Lys Asn Lys Met Ser Glu Ser Pro Ala Lys Thr Val Ser
 225 230 235 240
 Glu Glu Lys Ala Lys Gln Tyr Leu Glu Glu Phe His Gln Thr Ala Leu
 245 250 255
 Glu His Pro Glu Leu Ser Glu Leu Lys Thr Val Thr Gly Thr Asn Pro
 260 265 270
 Val Phe Ala Gly Ala Asn Tyr Ala Ala Trp Ala Val Asn Val Ala Gln
 275 280 285
 Val Ile Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Thr Ala Ala
 290 295 300
 Leu Ser Ile Leu Pro Gly Ile Gly Ser Val Met Gly Ile Ala Asp Gly
 305 310 315 320
 Ala Val His His Asn Thr Glu Glu Ile Val Ala Gln Ser Ile Ala Leu
 325 330 335
 Ser Ser Leu Met Val Ala Gln Ala Ile Pro Leu Val Gly Glu Leu Val
 340 345 350
 Asp Ile Gly Phe Ala Ala Tyr Asn Phe Val Glu Ser Ile Ile Asn Leu
 355 360 365
 Phe Gln Val Val His Asn Ser Tyr Asn Arg Pro Ala Tyr Ser Pro Gly
 370 375 380
 His Lys Thr Gln Pro Phe Leu Pro Trp Asp Ile Gln Met Thr Gln Thr
 385 390 395 400
 Thr Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys
 405 410 415

FIG. 20A

Arg Ala Ser Gln Asp Ile Arg Asn Tyr Leu Asn Trp Tyr Gln Gln Lys
 420 425 430
 Pro Asp Gly Thr Val Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His
 435 440 445
 Ser Gly Val Pro Ser Lys Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
 450 455 460
 Ser Leu Thr Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe
 465 470 475 480
 Cys Gln Gln Gly Asn Thr Leu Pro Trp Thr Phe Ala Gly Gly Thr Lys
 485 490 495
 Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 500 505 510
 Gly Gly Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
 515 520 525
 Pro Gly Ala Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe
 530 535 540
 Thr Gly Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu
 545 550 555 560
 Glu Trp Met Gly Leu Ile Asn Pro Tyr Lys Gly Val Ser Thr Tyr Asn
 565 570 575
 Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
 580 585 590
 Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val
 595 600 605
 Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser Asp Trp Tyr Phe
 610 615 620
 Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
 625 630 635 640
 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met
 645 650 655
 Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr
 660 665 670
 Ile Ser Cys Arg Ala Ser Gln Asp Ile Arg Asn Tyr Leu Asn Trp Tyr
 675 680 685
 Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile Tyr Tyr Thr Ser
 690 695 700
 Arg Leu His Ser Gly Val Pro Ser Lys Phe Ser Gly Ser Gly Ser Gly
 705 710 715 720
 Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala
 725 730 735
 Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp Thr Phe Ala Gly
 740 745 750
 Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly
 755 760 765
 Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu
 770 775 780
 Leu Val Lys Pro Gly Ala Ser Met Lys Ile Ser Cys Lys Ala Ser Gly
 785 790 795 800
 Tyr Ser Phe Thr Gly Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly
 805 810 815
 Lys Asn Leu Glu Trp Met Gly Leu Ile Asn Pro Tyr Lys Gly Val Ser
 820 825 830

FIG. 20B

Thr Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Lys
835 840 845
Ser Ser Ser Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp
850 855 860
Ser Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser Asp
865 870 875 880
Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Leu Thr Val Phe Ser
885 890 896

FIG. 20C

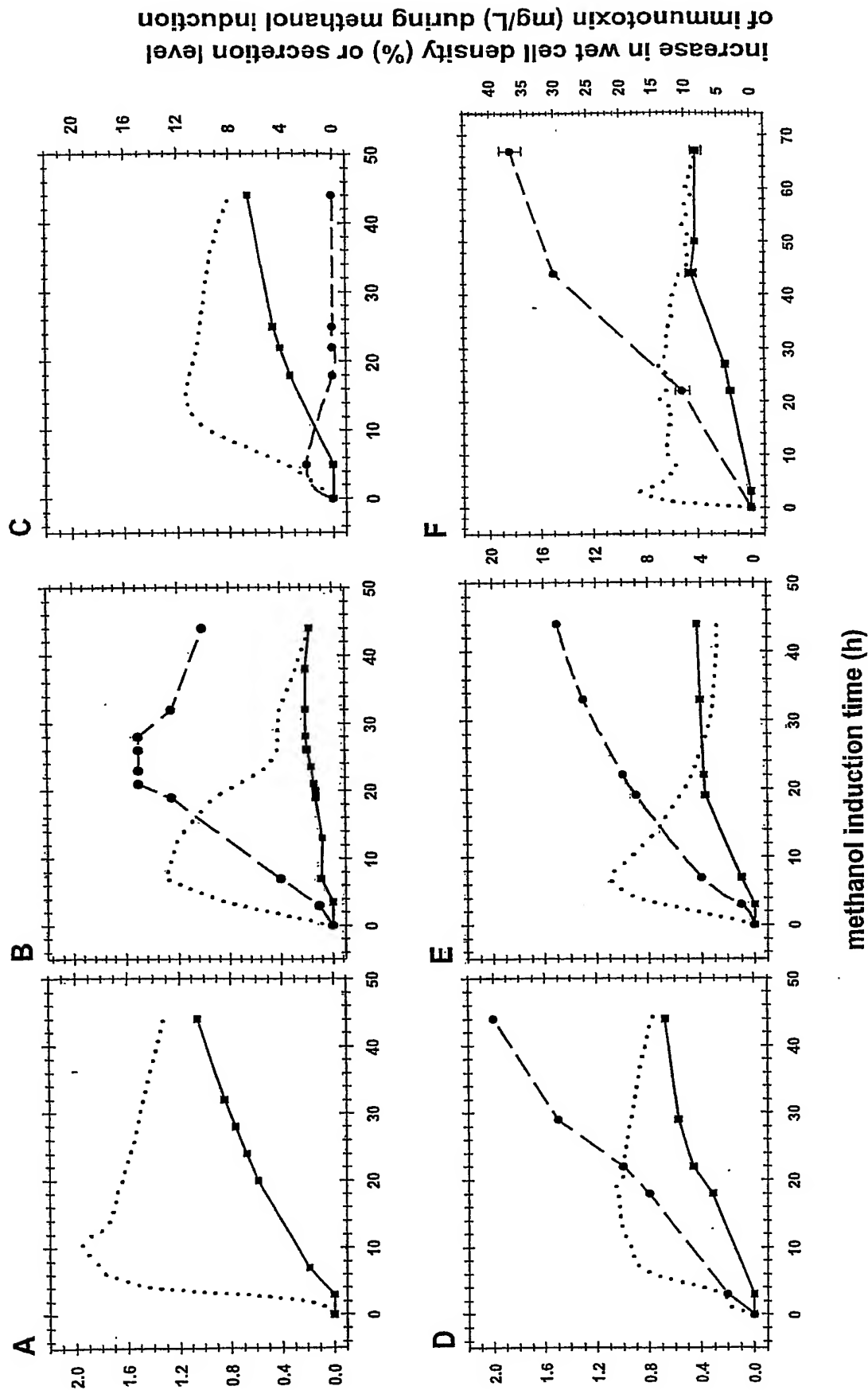
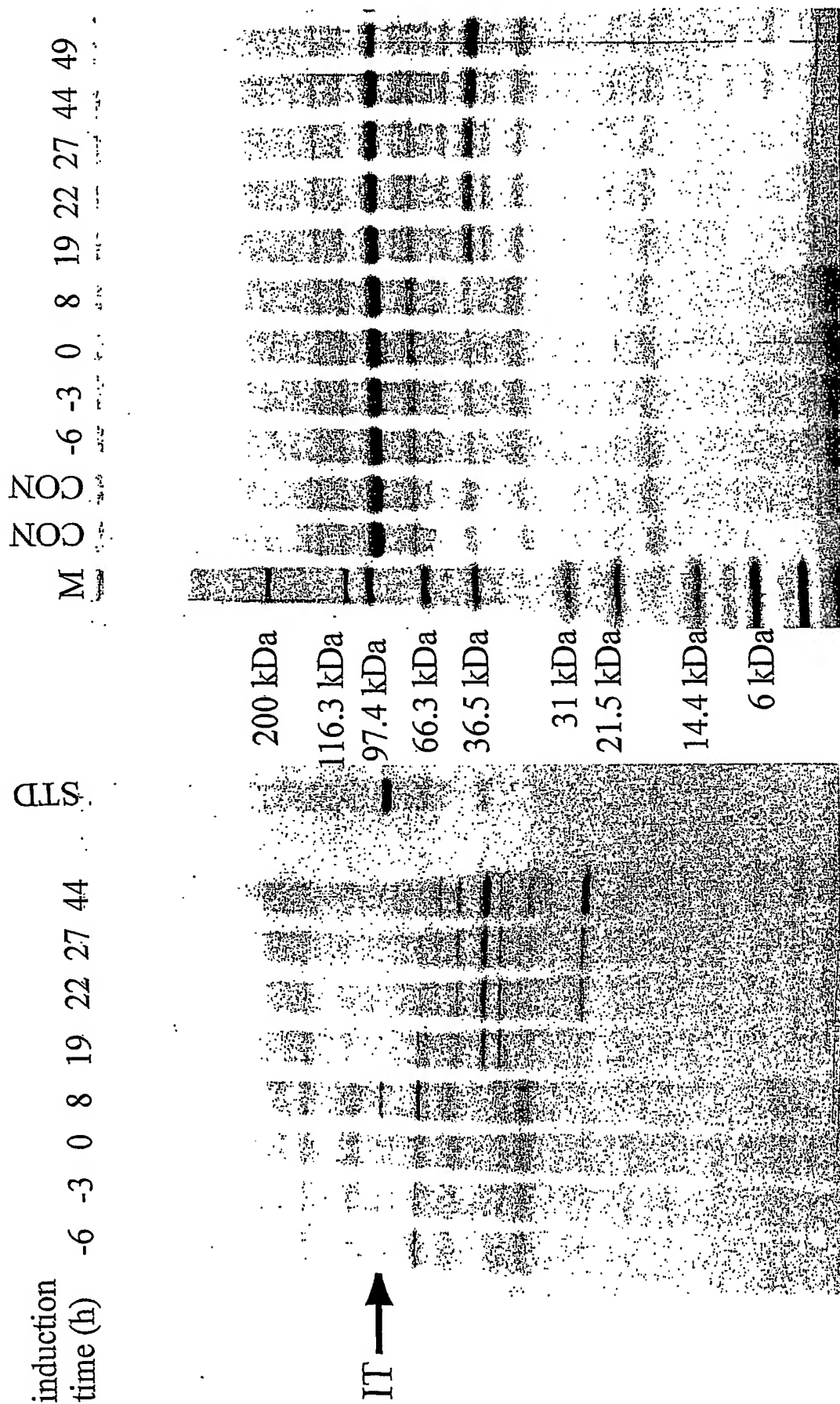


FIG. 21

B

FIG. 22



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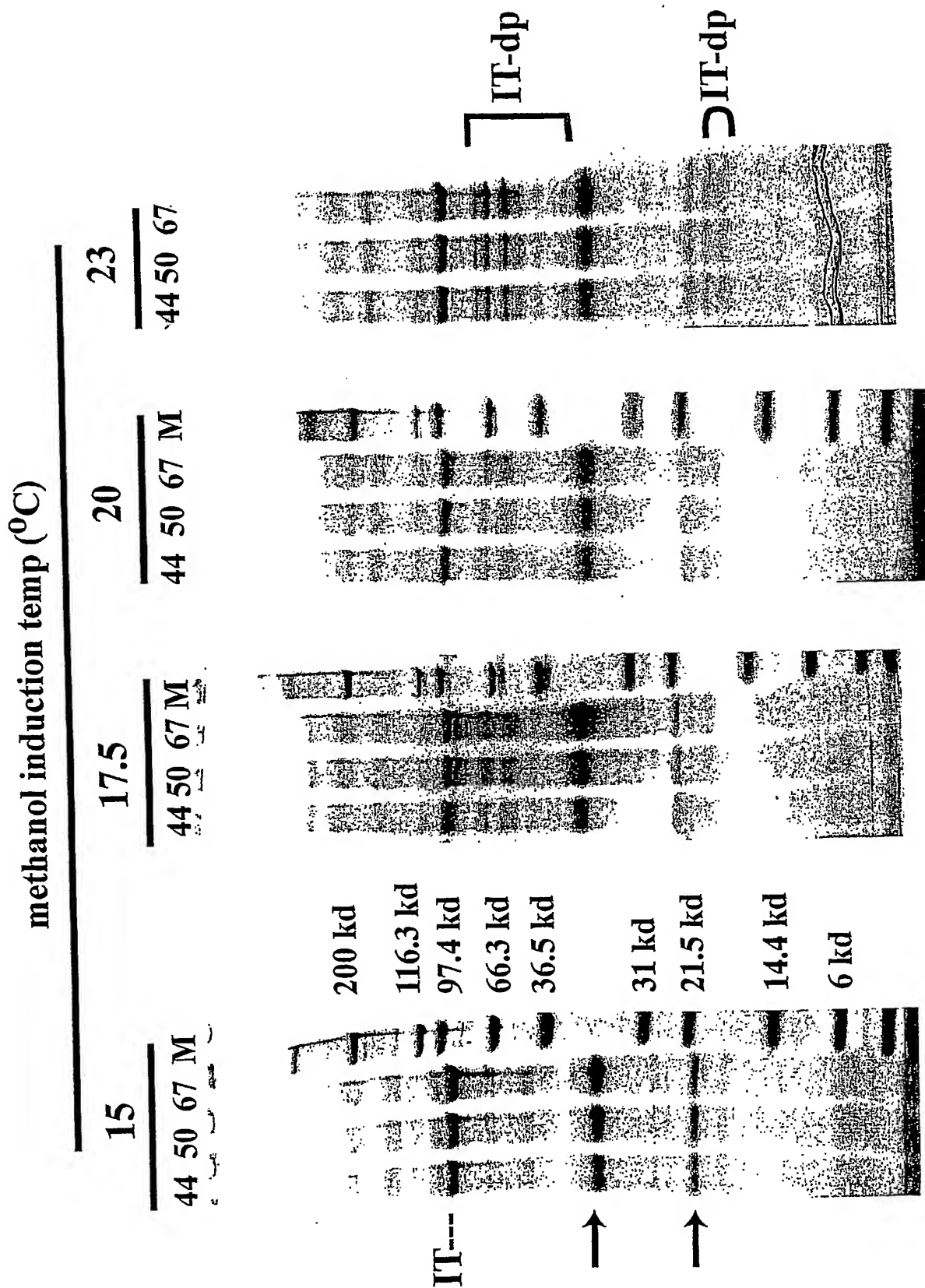


FIG. 3
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